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Radiobiology of the Newt, *Diemictylus viridescens*. Hematological and Histological Effects of Whole-body X Irradiation[1]

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(Plates I-III; Text-figure 1)

Introduction

In attempts to alter the course of pseudoerythroplastic anemia, a condition of the red cells found only in *Diemictylus viridescens* to date, (Jakowska & Nigrelli, 1952), newts were exposed to various amounts of X irradiation. Preliminary observations indicated that these animals survived for a comparatively long time after such treatments and that the normal and abnormal peripheral blood elements showed only slight changes even though the hemopoietic and other organs were extensively damaged.

Various species of amphibians have been used in radiobiological investigations, but we are not aware of any work relating to the radiation pathology of the newt, a salamander often used in studies on development, regeneration and hemopoiesis. The present studies, therefore, were made to determine the extent and nature of the radiation damage and, if possible, to indicate the factors that may be responsible for the apparently high degree of resistance of these animals to X irradiation.

Materials and Methods

Large adult male *Diemictylus viridescens* (= *Triturus viridescens*), weighing from 2.5 to 3.5 gm., obtained from Illinois, were used. Groups of ten animals each were exposed to X rays at room temperature (22-23° C.) in a specially constructed circular lucite container with a total of ten compartments, in which each animal was confined during treatment to a wedge-shaped area. The radiation factors were as follows: 250 kv; 30 ma; 0.5 mm. Cu and 1 mm. Al filters; target distance 40 cm.; dose rate 140-160 r/min. The doses administered were: 200, 950, 1700, 2450, 3200, 3950 and 4700 r for the animals kept at room temperature following irradiation. Doses of 200 and 1400 r were given to other groups used for experimental infection with *Pseudomonas hydrophila* and *Mycobacterium ranae*, and doses of 200 and 1700 r to animals with confirmed pseudoerythroplastic anemia. Other groups of animals, subsequently stored at 5° C., received 1700, 3000, 4700, 6000 and 9000 r. Following irradiation, all newts were maintained unfed in individual plastic containers, each with a small volume of tap water.

Mortality was recorded daily and random samples of blood and tissues from representative groups were obtained from sacrificed newts at weekly intervals, unless stated otherwise. Films stained with Wright's method were examined for specific absence of or increase in a cell type, or specific damage to cells. Differential counts were made from a number of cases. Paraffin sections of liver, kidney, spleen, intestine, testis, lungs and skin were stained with Hematoxylin-eosin, Giemsa, Masson's and Maximow's techniques.

Observations

Post-irradiation Behavior

Irradiated newts showed no cataracts, color changes or excessive skin shedding over the period of the observations. The animals were lively when aroused and demonstrated excellent muscle tonus even shortly before death. Treated animals differed from controls in that they spent more time out of water, climbing on the sides of the containers and remaining on the walls in a

[1] A portion of this work was carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

vertical position. They exhibited a mild hyperemia of the throat region and a very pronounced "buccal-pump" respiratory action, which was characterized by a rapid movement of the floor of the mouth. Both non-irradiated controls and irradiated newts stored at 5° C. remained lethargic, with some tendency to climb. Otherwise their condition appeared good, even five months following exposure to heavy doses of X rays.

Survival at Room Temperature

There was no significant statistical[2] difference in the survival of well-fed newts or those after a preliminary fast of one month, when exposed to 3200 r. With the assumption that the highest dosages act similarly in these unfed animals, the results of two sets of experiments were considered collectively. Statistical analysis showed highly significant differences between doses and a highly significant linear trend. The linear relationship between dose and number of days of survival fitted the equation $y = 24.8 - .003432 (x - 3008)$. The above equation was used to determine the LD 50/30 days for 50 of the treated animals kept at room temperature and was found to be 1486 r. The animals which received 200 r survived for four months or more at room temperature and the 200 r dose was omitted from the analysis.

Survival at 5° C.

Animals exposed to 1700, 3000, 6000 and 9000 r and kept at 5° C. remained alive for as long as five months. The mortality was negligible and the animals selected at random to be sacrificed appeared in good condition, although they remained lethargic in the cold room most of the time. These newts were large and robust and apparently in an excellent nutritional state when they were irradiated. After remaining unfed for five months at 5° C., most animals still had large fat-bodies and fat deposits around the cloaca. Blood was abundant and normal in color; the liver was firm, but the spleen paler and more fragile than in the controls; the lungs were pinkish and partly edematous.

Peripheral Blood

The blood of the irradiated newts did not clot as rapidly as that of the controls although no accurate data are available. This may have been related to the abnormalities observed in the thrombocytes; these were swollen, had uneven cellular borders, and stained atypically with poor differentiation of the nucleus and of cytoplasmic granules, within one week following a dose of 200 r. This condition of the thrombocytes was consistently seen throughout the life of the irradiated animals regardless of dosage (Pl. I, Fig. 1).

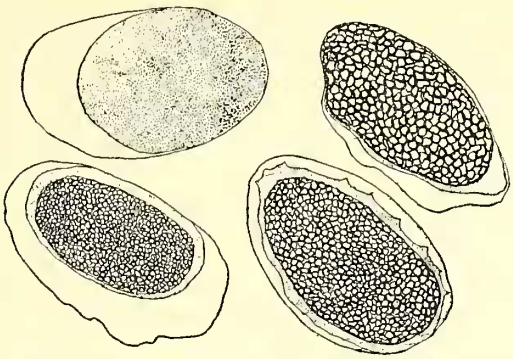
Neutrophils were studied from the peripheral blood and in sections of the liver, which is the chief site of their production. The animals kept at room temperature for 19 days after 200 and 1400 r were challenged with *Pseudomonas hydrophila* and *Mycobacterium ranae* to determine their ability to ward off infections (Jakowska, Nigrelli & Sparrow, 1954). Active phagocytosis, with the participation of numerous neutrophilic leukocytes, was observed 24 hours after the infection was induced on the surface of the tail.

Some neutrophilia, with Classes V and VI predominating, appeared, but only in newts exposed to 200 r. With higher dosages the neutrophils in the animals kept at room temperature were not numerous at any time, but in those kept in the cold for three months following 1700 r or more, obvious neutrophilia with banded (non-segmented) forms predominating was noted (Jakowska & Nigrelli, 1954), together with an increase in the number and in the size of the basophilic granulocytes (Pl. I, Fig. 2). No eosinophils were seen in the peripheral blood of irradiated newts, although these were occasionally seen in the controls.

The erythrocytes did not appear affected, and only occasionally were nuclear fragments seen with a frequency higher than normal (Pl. I, Fig. 3). The nuclear chromatin pattern of the erythrocytes was not basically altered, except in the animals kept for three months in the cold and exposed to the highest dose (9000 r). In these animals the nuclear contents of the erythrocytes appeared somewhat less distinct. Cellular elements of the blood appeared normal in the few newts kept for five months at 5° C. following 6000 r treatment. There was no significant change in the number of erythroplastids (non-nucleated red elements) and microcytes in irradiated animals.

Pseudoerythroplastic anemia (Jakowska & Nigrelli, 1952) was found sporadically in irradiated and non-irradiated unfed newts (Pl. I, Fig. 4). Effects of X irradiation (200 and 1700 r) on the pseudoerythroplastids were studied by exposing such anemic animals. Twenty-four hours after 1700 r, the pseudoerythroplastids of the peripheral blood showed a characteristic separation of the cytoplasm from the cell membrane, possibly indicating that their permeability has been affected (Text-fig. 1). Counts of the early, intermediate and late stages of pseudoerythroplastids before and 24 hours after irradiation failed to reveal numerical differences.

[2] For statistical analysis we are indebted to Dr. Phelps P. Crump of the Brookhaven National Laboratory.



TEXT-FIG. 1. Pseudoerythroplastids of an anemic newt, 24 hours after exposure to 1700 r. Note the separation of the cell membrane from the cytoplasm. From Wright's stained smear. 4000 ×.

Blood Parasites

Blood parasites were occasionally seen in control and irradiated newts. The cytological appearance of trypanosomes seemed normal even in animals exposed to 4700 r. The trypanosomes also occurred in the blood of some irradiated newts kept in the cold for five months. An exceptionally high degree of infection with *Babesiosoma* (*Dactylosoma*) *jahni* (Jakowska & Nigrelli, 1956), a non-pigmented haemosporidian, was found in one animal treated with 950 r and kept at room temperature.

Liver

Progressive degeneration of the liver was observed in the animals kept at room temperature. Three weeks after 2450 r, for example, the nuclei of the hepatic cells were pyknotic and the granulocyte-forming perihepatic layer was considerably reduced in thickness and activity. Numerous vacuolated areas in the hepatic cells indicated lipid accumulation. In the animals kept in the cold, the degeneration of the parenchyma was slower but more conspicuous. The perihepatic layer itself was not essentially affected with the lower doses (1700 r) and retained its usual thickness and mitotic activity for about three months (Pl. I, Fig. 5). One month after 4700 r, however, the hepatic capsule was reduced to a thickness of one cell, although the nuclei of the liver cells appeared normal. Exposure to 6000 and 9000 r within one month was followed by the formation of numerous hyalin droplets in the cytoplasm of the parenchyma cells (Jakowska & Nigrelli, 1954). These droplets stained red with Masson and light blue with Giemsa (Pl. II, Fig. 6).

Some recovery in the liver parenchyma was apparent in animals kept for four months in the cold after exposure to higher doses. The cytoplasm was finely granular, and, as in the controls, the nuclei did not show pyknosis, but some seemed to contain more than the usual number of nucleoli. A few young leukocytes and occasional macrophages were found scattered in the parenchyma.

Kidney

The kidney was very firm in the animals surviving 3000, 6000 and 9000 r for three months or longer in the cold. In animals kept at room temperature, cloudy swelling was seen in the tubular epithelium of the kidney within two to three weeks following 1700 r. The changes progressed further in the animals kept alive for longer periods at 5°C. "Albuminous" material accumulated in the lumen of the tubules (Pl. II, Fig. 7) and in the glomeruli. Later, hyalin droplets were found in the cytoplasm of the cells of the urinary ducts (Pl. II, Fig. 8) and of tubules. These droplets stained similarly to those found in the liver cells (Jakowska & Nigrelli, 1954). The tubular lumen was occluded by homogeneous basophilic material and the lining of the urinary ducts had often been sloughed.

Spleen

The spleen of irradiated newts kept at room temperature underwent gradual atrophy. In the animals stored at 5°C., on the other hand, the size of the spleen was consistent with that of non-irradiated controls, showing similar individual variations. Sections, however, revealed large hemosiderin deposits and many destroyed red blood cells. There was a reduction in the hemopoietic tissue and the development of dense fibrous material. Infrequently, a spleen of semi-fluid consistency with a thick fibrous capsule was found (Pl. III, Fig. 9). Such spleens were especially pale in those animals which had received 6000 r, but which had abundant bright red, freely flowing blood. No active erythropoiesis or thrombocytopoiesis were observed. Large numbers of basophilic granuloctyes were present.

Intestine

Irradiated animals remained unfed throughout the period of observations. There were no changes in the intestinal epithelium indicative of ulcerative damage, nor any desquamation. The margins of the cells of the intestinal mucosa appeared intact. No hemopoiesis was observed in the submucosa.

Testes

Animals that were kept for four months in the cold following 3000, 6000 and 9000 r showed no evidence of spermatogenesis, with the majority of the cells pyknotic and with very

little interstitial tissue (Pl. III, Fig. 10). Moderate spermatogenesis was found in the testicular lobes of cold-stored controls.

Lungs

The pulmonary vessels of the irradiated animals showed very little blood. There was a profuse exudate in the lungs of newts kept for one month at 5°C. following the highest doses of X irradiation. Even at lower doses, *e.g.* 1700 r after three months in the cold, exudate containing numerous basophilic granulocytes with coarse irregular granules and microcytes was observed in the lungs. *Hexamita*-like flagellates were occasionally found in such edematous lungs (Pl. III, Fig. 11).

Skin

In view of the osmo-regulatory function of the skin in the adult newt, particular attention was given to this organ. Controls kept at room temperature showed an epithelial layer two or three cells in thickness, a highly vascularized corium and actively secreting dermal glands. Controls kept in the cold had thickened epithelium, numerous cells in mitosis, a corium with constricted capillaries and dermal glands filled with secretory granules. Newts exposed to the highest doses of X irradiation and kept in the cold for three months showed slight increase in thickness of the epithelium, no mitosis and considerable keratinization (Pl. III, Fig. 12). There was an increase in the number of dermal glands, associated with an increased stickiness of the body, a condition which disappeared at four and five months. Shedding in all irradiated animals was repeated but not excessive; it occurred in the form of small fragments instead of the usual large epithelial sheets.

Discussion

Delayed radiation effects in amphibians were reported by Stearner (1950) for *Rana pipiens* and by Brunst, Sheremetieva-Brunst & Figge (1953) for the axolotl. The absence of early manifestations was attributed to the lower metabolic rate of these cold-blooded animals, or to the greater potential for regeneration.

The data on survival of irradiated newts at room temperature and at 5°C. compare favorably with the results of Patt & Swift (1948) on *Rana pipiens;* the degenerative effects of irradiation in newts were not as rapid as those reported for mammals in similar organs (liver, spleen, lung, intestine, skin, testis, etc.).

Bacteremia was absent at all stages after irradiation in newts. It was apparent that these animals still had ability to mobilize phagocytic neutrophils, as demonstrated by the response of these elements to experimentally induced infection (Jakowska, Nigrelli & Sparrow, 1954). This is significantly different from the typical acute radiation syndrome in mammals, where fatal infection characterized by a low granulocyte count plays the predominant role (Cronkite & Bond, 1956).

From the present studies, it was evident that the irradiated newts were able to live for a comparatively long time in spite of damage to several vital organs, *viz.* kidney, liver, lung and spleen. It is well known that cold-blooded animals, especially fish and amphibians, can survive extensive destruction of renal tissue, because a certain amount of osmo-regulation takes place in the gills and skin. Further, it has been shown that newts lived indefinitely with both lungs removed (Philippi, Hausler, Bialy & Jakowska, 1956; Jakowska, Philippi & Nigrelli, 1956). Since considerable skin damage occurred in the irradiated newts, it may be assumed that death was due to the impaired efficiency of the skin in both respiratory and osmo-regulatory functions. Histologically, however, the damage did not involve the formation of abnormal epithelium, as reported by Brunst (1955) in irradiated tadpoles of *Rana catesbeiana.*

In spite of the fact that the principal hemopoietic sites, *e.g.* the granulocytopoietic layer of the liver and the thrombo- and erythrocytopoietic spleen, were severely injured, the peripheral blood picture remained relatively unaffected. This fact, together with the absence of any mitotic activity in the cells of the peripheral circulation, was indicative of the comparatively long life (approximately 150 days at 5°C.) of the blood elements in this species. The delayed clotting in irradiated newts was probably related to the damage observed in the thrombocytes. The presence of increased numbers of giant basophilic granulocytes, seen in the peripheral blood of newts exposed to 1700 r and kept in the cold for three months or longer, may also have some relationship to the delayed clotting, since it has been shown that basophilic granulocytes (mast cells) in other vertebrates contain heparin (Wilander, 1939; Veil & Quivy, 1950).

Summary

Adult newts (*Diemictylus viridescens*) were relatively resistant to high doses of X irradiation, surviving for up to five months following exposure to 9000 r when kept at 5°C. after X ray treatment. At room temperature of 22-23°C., the LD 50/30 days was found to be 1486 r.

There was no appreciable effect on the blood elements of the peripheral circulation, although there was extensive damage to the hemopoietic

organs (erythrocyte- and thrombocyte-forming spleen and granulocyte-forming layer of the liver). No bacteremia was found at any stage and irradiated newts showed the ability to ward off experimental infection by active phagocytosis.

The above may be related to the radioresistance of this species. It is assumed that death was due to the destruction of the osmo-regulatory functions in organs such as the kidney, lungs and skin.

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Bibliography

Brunst, V. V.
1955. The reaction of the skin epithelium of the tails of *Rana catesbiana* tadpoles to local X irradiation. Radiation Research, 2 (6): 556-567.

Brunst, V. V., E. A. Sheremetieva-Brunst & Frank H. J. Figge
1953. A comparison of the reactions of the irradiated parts of the bodies of two-day old mice and urodele amphibians to Roentgen treatment. Am. J. Roentgen. Radium Ther. and Nucl. Med., 70 (2): 283-293.

Cronkite, E. P., & V. P. Bond
1956. Effects of radiations on mammals. Annual Rev. of Physiol., 18: 483-526.

Jakowska, Sophie, & R. F. Nigrelli
1952. Further studies on anemia in *Triturus viridescens*. Caryologia, 4: 281-288.

Jakowska, Sophie, R. F. Nigrelli & A. H. Sparrow
1954. Local cell responses to induced skin infection in *Triturus viridescens* after whole-body irradiation. Radiation Research, 1 (6): 569.

Jakowska, Sophie, & R. F. Nigrelli
1954. Histopathologic changes in *Triturus viridescens* after X irradiation with survival prolonged by low temperature. Anat. Rec., 120 (3): 746.
1956. *Babesiosoma* gen. nov. and other babesioids in erythrocytes of cold-blooded vertebrates. Ann. N. Y. Acad. Sci., 64: 112-127.

Jakowska, Sophie, C. Philippi & R. F. Nigrelli
1956. Blood studies on pneumectomized and splenectomized newts (*Diemictylus viridescens*). Anat. Rec., 125 (3): 645.

Patt, H. M., & M. N. Swift
1948. Influence of temperature on the response of frogs to X radiation. Am. J. Physiol., 155: 388-393.

Philippi, C., L. Hausler, H. Bialy & Sophie Jakowska
1956. Survival of newts (*Diemictylus viridescens*) following lung ligation and extirpation. Anat. Rec., 125 (3): 656.

Stearner, S. Phyllis
1950. The effects of X irradiation on *Rana pipiens* (Leopard frog) with special reference to survival and to the response of the peripheral blood. J. Exptl. Zool., 115: 251-262.

Veil, C., & D. Quivy
1950. Sur la présence d'héparine dans les écailles de la Carpe. C. R. Soc. Biol., 144: 1483.

Wilander, O.
1939. Studien über Heparin. Scand. Arch. Physiol. 81: 1-89.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. Damaged thrombocytes in the blood of an animal kept for five months at 5°C. following exposure to 6000 r. Wright's stain. 1800 ×.

Fig. 2. Giant basophilic granulocyte (somewhat flattened), in the blood of a newt kept for three months at 5°C. following exposure to 1700 r. Wright's stain. 1000 ×.

Fig. 3. Erythrocyte with two small, unequal nuclear fragments, found in the blood of a newt kept for one month at 5°C. following exposure to 1700 r. Wright's stain, 2200 ×.

Fig. 4. Normal erythrocytes and pseudoerythroplastids (two early stages are seen in the upper part of the picture and one fully formed, showing surface damage, below center) in the peripheral blood of a newt kept for one month at 5°C. following exposure to 9000 r. Wright's stain. 1800 ×.

Fig. 5. Granulocytopoietic layer of the liver, with several cells appearing to be in mitosis, from a newt kept for three months at 5°C. following 1700 r. Maximow's stain. 900 ×.

Plate II

Fig. 6. Hyalin droplets in the liver cells of a newt kept for one month at 5°C. following exposure to 6000 r. Masson's trichrome stain. 900 ×.

Fig. 7. Degeneration of the kidney tubules in a newt kept for one month at 5°C. following 9000 r. Masson's stain. 600 ×.

Fig. 8. Accumulation of hyalin droplets in the cells of the urinary duct and the occlusion by hyaline material in a newt kept for two months at 5°C. following exposure to 1700 r. Masson's stain. 900 ×.

Plate III

Fig. 9. Spleen damage in a newt kept for four months at 5°C. following 9000 r. Hematoxylin-eosin stain. 450 ×.

Fig. 10. Testis of the same newt as in Fig. 9. Note pyknosis and absence of spermatogenesis. Hematoxylin-eosin stain. 450 ×.

Fig. 11. Abnormal basophilic granulocyte, a microcyte and a flagellate found in the lung exudate of a newt kept for three months at 5°C. following 1700 r. Wright's stain. 2000 ×.

Fig. 12. Skin of a newt exposed to 6000 r and kept in the cold for one month. Note the keratinization of the epidermis. Masson's stain. 450 ×.